Molecular Epidemiology of Pulmonary Tuberculosis in Southern Ethiopia

Yared Merid (✉ meridyared@gmail.com)

Hawassa University College of Medicine and Health Sciences <https://orcid.org/0000-0002-5885-4119>

Elena Hailu

Armauer Hansen Research Institute

Getnet Habtamu

Armauer Hansen Research Institute

Melaku Tilahun

Armauer Hansen Research Institute

Markos Abebe

Armauer Hansen Research Institute

Mesay Hailu

Hawassa University College of Medicine and Health Sciences

Tsegaye Hailu

Armauer Hansen Research Institute

Daniel G. Datiko

Management Sciences for Health, Ethiopia

Yimtubezinash Woldeamanuel

Addis Ababa University, College of Health Sciences

Abraham Aseffa

Armauer Hansen Research Institute

Research article

Keywords: Molecular epidemiology, Drug susceptibility testing, Genotypic cluster, Ethiopia

Posted Date: February 17th, 2020

DOI: <https://doi.org/10.21203/rs.2.23681/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Understanding the epidemiology of tuberculosis (TB) is limited by lack of genotyping data. We sought to characterize the drug susceptibility testing (DST) patterns and genetic diversity of *M. tuberculosis* (Mtb) isolates in Southern Ethiopia.

Methodology

A cross-sectional study was conducted among newly diagnosed sputum smear positive patients with TB visiting nine health facilities in southern Ethiopia from June 2015 to May 2016. Three consecutive sputum samples (spot-morning-spot) per patient were examined using acid-fast bacilli (AFB) smear microscopy with all smear positive specimens having AFB cultures performed. Mtb isolates had DST performed using indirect proportion method and were genotyped with RD9 deletion typing and spoligotyping. Spoligotyping International Types (SIT) and sub-lineages (clades) were assigned according to the SITVITWEB data base.

Results

Among 250 newly diagnosed patients with TB, 154 (52%) were male and 143 (57%) from rural areas. The prevalence of HIV co-infection was 4%. Of the 250 AFB positive sputum samples, 230 (92%) were culture positive. All 230 isolates were *M. tuberculosis* strains belonging to three lineages: Euro-American, 187 (81%); East-African-Indian, 31 (14%); and Lineage 7 (Ethiopian lineage), 8 (4%). The 230 isolates could be categorized into 65 different spoligotype patterns, of which 84% fell into 29 clusters. The dominant spoligotypes were SIT149 (21%), SIT53 (19%) and new strains (16%). Mtb strains were clustered by districts. DST revealed that 14% of Mtb isolates were resistant to > 1 first line anti-TB drugs including 11% to isoniazid. SIT 149 was the most prevalent genotype among drug resistant isolates (20%). Conclusion

The study revealed several clusters including lineage 7 strains circulating in southern Ethiopia. SIT 149 (T3-ETH) was the most dominant circulating strain in the study area including among drug-resistant cases.

Background

Tuberculosis (TB) remains a major and urgent global public health problem, especially in low-income countries where the burden of disease is high. Globally, there were 10 million new cases and 1.5 million deaths among persons with TB in 2017 (1). The spread of TB is fueled by several factors including the HIV epidemic, low socioeconomic status, overcrowding, malnutrition, and the emergence of drug-resistance (2–4).

Drug-resistant TB is a major public health concern in many countries and continues to be a public health crisis (1). Ethiopia is one of fourteen countries appearing in all three WHO high burden country lists for

TB, TB/HIV and MDR-TB. According to the national anti-tuberculosis drug resistance survey in 2014, the prevalence of MDR-TB among new and previously treated TB cases was 2.3% and 17.8% respectively (5). WHO estimated the prevalence of MDR-TB among new TB cases in Ethiopia at 2.7% and among previously treated cases at 14% in 2017 (1). Additionally, data from different parts of the country show that drug-resistant TB is a major public health concern that demands attention (6–8).

Interruption of the transmission of *M. tuberculosis* is one of the primary goals of tuberculosis control programs. Tracking specific strains of *M. tuberculosis* circulating in the community informs public health authorities on patterns of spread and potential areas for action to curb the spread of TB in communities (9). Spoligotyping is a PCR-based method commonly used to characterize of *M. tuberculosis* strains circulating in a community (10) and strain differentiation is based on the polymorphism in the direct repeat (DR) locus, which is a distinct chromosomal region in *Mtb* genome (11).

While several molecular epidemiological studies have been conducted to describe the diversity and drug susceptibility profile of *M. tuberculosis* strains in various geographical areas of Ethiopia (7, 12–14), data from the south, where population density is relatively higher, is limited. The goal of our study was to characterize the drug susceptibility patterns and genetic diversity of *M. tuberculosis* isolates circulating in southern Ethiopia.

Materials And Methods

Study setting/design

This cross-sectional study was conducted at nine health facilities (two hospitals and seven health centers) in and around Shashemene area, in West Arisi Zone of Oromia Region, Ethiopia. Shashemene is a major urban center and commercial town, located 240 km south of the capital Addis Ababa. The estimated population of the study area including of Shashemene and the adjacent rural towns of Wondo Genet, Aje, and Arsi-Negele was 3 million people. All nine health facilities included in the study area provided services for the diagnosis and treatment of TB through DOTS clinics. Patients enrolled at the nine health facilities from the West Arsi Zone and adjoining kebele of Wondo Genet were mapped and fell into seven districts (Woredas) (Fig. 1).

Study population and variables

Among the persons suspected to have TB who were investigated at any one of the nine health facilities during the study period, all newly diagnosed sputum smear positive pulmonary TB patients who provided written informed consent for study participation were enrolled. Assent for the children and consent by parents or guardians for those under 18 yrs of age were also obtained. The study was conducted from June 2015 through May 2016. Three consecutive sputum samples (spot-morning-spot) were collected from each TB suspect. Socio-demographic and clinical information was obtained for all study participants by a TB clinic nurse at the respective health facilities using a pre-tested standard questionnaire. Data on HIV status was retrieved from health facility records.

Laboratory methods

Specimen Collection

Sputum smears were prepared and examined by an onsite health facility laboratory technologist. The remaining portion of the three sputum samples from AFB smear positive patients were pooled individually into 50 ml sterile screw capped universal test tubes and stored at the diagnostic centers at -20°C for a maximum of one week until transported on cold chain to the Armauer Hansen Research Institute (AHRI) in Addis Ababa, Ethiopia for mycobacterial culture and molecular testing.

Mycobacterial Culture

The pooled sputum samples were processed at AHRI within one day based on standard procedures as previously described (15). In brief, the sputum samples were digested and decontaminated using Petroff's method and the processed sample was inoculated into three tubes containing egg-based Löwenstein Jensen (LJ) media (two with glycerol and one with pyruvate). The inoculated media were incubated at 37°C for at least 8 weeks, with weekly observation for the presence of mycobacterial colonies. Cultures with no growth after the eighth week were considered negative. Mycobacterial growth was confirmed by typical colony morphology and AFB staining.

Drug Susceptibility Test (DST)

The conventional indirect proportion method was employed to perform DST using 7H10 medium on 24-well tissue culture plates using a standard protocol (16). In brief, four first line anti-TB drugs (isoniazid, rifampicin, ethambutol and streptomycin) were mixed with the agar media at recommended concentration and dispensed into 9 wells of the 24 well tissue culture plates and two wells were dispensed with drug free media. The agar plate was sealed with parafilm and incubated in an inverted position at 35°C. The plates were checked on day 6, 12 and 19 for evidence of growth. Resistance was expressed as the percentage of colonies that grew on critical concentrations of the drugs, i.e. 0.2 µg/ml for isoniazid (INH), 1 µg/ml for rifampicin (RPM), 5 µg/ml for ethambutol (EMB) and 2 µg/ml for streptomycin (STM). The interpretation of resistance was based on the standard criteria for resistance, i.e. 1% for all drugs (17).

RD9 Deletion Analysis

Region of difference 9 (RD9) deletion analyses was performed on heat-killed cells to confirm the presence or absence of RD9 for species identification of *M. tuberculosis* from the other members of MTBC as previously described (18). It uses three primers (RD9flankF, RD9intR and RD9flankR) for PCR reaction and the PCR amplification product was run by electrophoresis in 1.5% agarose gel and the results were interpreted as *M. tuberculosis* when a band of 396 bp was observed (RD-9 positive). Detection of a band size of 575 bp was considered to be positive for the other members of *M. tuberculosis* complex species (*M. bovis* or *M. africanum*).

Spoligotyping

Isolates that were positive for *M. tuberculosis* by RD9 PCR were further characterized by spoligotyping following the procedure described earlier (10). In brief, the direct repeat (DR) region of the isolate was amplified by PCR using oligonucleotide primers (DRa and DRb) derived from the DR sequence. Individual spoligotyping patterns were compared with the recent International Spoligotyping Database (SITVITWEB). Spoligotyping International Types (SIT) and sub-lineages (clades) were assigned according to signatures provided in SITVITWEB data base (<http://www.pasteur-guadeloupe.fr:8081/SITVIT2/>). An isolate was defined as a shared type if the same spoligotype was found in the database. If no matching spoligotype was found in the database, the isolate was defined as orphan (new). Lineage types were assigned using a protocol for classification and analysis of MTBC (19).

Definitions

Newly diagnosed patients with pulmonary TB refer to patients who had never been previously treated for TB or have only taken anti-TB drugs for less than 1 month. *M. tuberculosis* isolates (two or more) that share the same genotype based on spoligotyping were considered clustered.

Data Management

All data were double entered into an online REDCap database (20) and analyzed using STATA software.

Ethical Consideration

The study was approved by the Institutional Review Boards of Addis Ababa University and the Armauer Hansen Research Institute (AHRI) as well as the Ethiopian National Ethics Review committee. Study permission was also obtained from the Oromia Regional Health Bureau, Western Arisi zone Health Department, Southern Regional Health Bureau and Sidama Zone Health Department.

Results

Socio-demographic characteristics

Among 250 newly diagnosed sputum smear positive TB patients enrolled, 145 (58%) were male and 143 (57%) from urban areas (Table 1). The median age was 25 years (interquartile range [IQR] 20–30). One hundred twenty-nine (52%) were married, 195 (76%) had an educational level of at least primary school. Farmers, students and house wives altogether accounted for 70% (174) of the study participants. TB-HIV co-infection was present in 10 (4%).

Table 1
Sociodemographic characteristics and clinical variables of
study participants

Characteristics	Number	Percentage (%)
Sex	145	52.0
Male	105	48.0
Female		
Age in years	14	5.6
<14	193	77.2
15–34	23	9.2
35–44	9	3.6
45–54	11	4.4
≥ 55		
Location	107	42.8
Urban	143	57.2
Rural		
Marital status	109	43.6
Single	129	51.6
Married	12	4.8
Other		
Education	195	78.0
Primary school and above	55	22.0
Illiterate		
Occupation	79	31.6
Farmer	57	22.8
Student	38	15.2
Housewife	9	3.6
Government employee	67	26.8
Other		
Clinical variables	213	85.5
Fever	220	88.0
Night sweat	223	89.2
Loss of appetite	232	92.8
Weight loss	208	83.2
Chest pain		
HIV serostatus	10	4.0
Positive	236	96.0
Negative	4	1.6
Not tested		

Genetic diversity of strains

All 250 AFB positive sputum samples had mycobacterial culture performed; 230 (92.0%) were positive, 8 (3.2%) were contaminated and 12 (4.8%) failed to grow on culture (Fig. 2). The 230 isolates were all identified as *M. tuberculosis* by RD9 deletion analysis. Spoligotyping analysis found a total of 65

spoligotype patterns, of which 36 (55.0%) were already known in the international data base and 29 (45.0%) were new patterns (orphans).

The lineage distribution showed that 187 (81%) isolates belonged to the Euro-American lineage (L4), 31 (14%) to East-African-Indian (L3) and 8 (4%) to Lineage 7 (Ethiopian lineage). Four strains could not be assigned to any of the lineages. The predominant clade (sub-lineage) was T1 (51, 22%), followed by T3-ETH (48, 21%), H3 and CAS1-Delhi (23, 10%) each.

The most dominant shared types were SIT149 (48, 21%) and SIT53 (44, 19%) and orphan strains (of different spoligotype patterns) (37, 16%). One hundred ninety-three (84%) isolates were clustered into 29 spoligotype patterns and the remaining (37, 16%) strains fell into single spoligotypes. Cluster size varied from 2 to 48 strains per cluster. Of the clustered 193 strains, 179 (93%) were already registered in the international data base and the other 14 (7%) were orphans. Sixty-two (27%) of the strains were not assigned for clade in the SITVITWEB database (Table 2).

Table 2
Spoligotyping result of 230 isolates, Southern Ethiopia

Lineages, Sub lineages (clades)	SIT (No. of samples. % of the total sample)
Lineage 4, T1 (n = 51)	SIT 53 (n = 44, 19.0%)
	SIT 358 (n = 4, 1.7%)
	SIT 334 (n = 2, 0.9%)
	SIT 205 (n = 1, 0.4%)
Lineage 4, T3- ETH (n = 48)	SIT 149 (n = 48, 20.9%)
Lineage 4, T2 (n = 2)	SIT 52 (n = 2, 0.9%)
Lineage 4, Ambiguous: T2 × 1 (n = 2)	SIT 336 (n = 2, 0.9%)
Lineage 4, T3 (n = 12)	SIT 37 (n = 10, 4.4%)
	SIT 121 (n = 2, 0.9%)
Lineage 4, Ambiguous: T3T2 (n = 1)	SIT 73 (n = 1, 0.4%)
Lineage 4, H3 (n = 23)	SIT 134 (n = 1, 0.4%)
	SIT 49 (n = 1, 0.4%)
	SIT 669 (n = 10, 4.4%)
	SIT 390 (n = 2, 0.9%)
	SIT 394 (n = 2, 0.9%)
	SIT 777 (n = 7, 3.0%)
Lineage 4, H4 (n = 1)	SIT 35 (n = 1, 0.4%)
Lineage 4, LAM10-CAM (n = 1)	SIT 838 (n = 1, 0.4%)
Lineage 4, sub-family not assigned (n = 47)	SIT 4 (n = 5, 2.2%)
	SIT 159 (n = 1, 0.4%)
	SIT 156 (n = 4, 1.7%)
	SIT 46 (n = 3, 1.3%)
	SIT 1410 (n = 2, 0.9%)
	SIT 602 (n = 3, 1.3%)
	Orphan (n = 29, 12.6%)
Lineage 3, CAS1-Delhi (n = 23)	SIT 25 (n = 6, 2.6%)

Lineages, Sub lineages (clades)	SIT (No. of samples. % of the total sample)
	SIT 26 (n = 11, 4.8%)
	SIT 247 (n = 1, 0.4%)
	SIT 1198 (n = 1, 0.4%)
	SIT 1314 (n = 1, 0.4%)
	SIT 2359 (n = 2, 0.9%)
	SIT 485 (n = 1, 0.4%)
Lineage 3, CAS2 (n = 1)	SIT 1979 (n = 1, 0.4%)
Lineage 3, sub-family not assigned (n = 7)	Orphan (n = 7, 3.0%)
Lineage 7	SIT 910 (n = 8, 3.5%)
Lineage unknown, MANU2 (n = 2)	SIT 54 (n = 2, 0.9%)
Lineage not assigned (n = 4)	No described patterns (n = 3, 1.3%) Orphan (n = 1, 0.4%)

Mapping of TB lineage and strain clusters

The spatial distribution of lineages identified in the study is presented in Fig. 3.

Mapping of the geographic location of clustered strains showed that the distribution of clustered strains varies within districts in the study area, and the highest proportions of clustered strains were observed in Wondo Genet district of Sidama Zone with SIT 149 (6.5%), SIT 699 (3.0%), and SIT 25 (2.6%). Shashemene town also had a higher proportion of clustered strains such as SIT 149 (4.4%), SIT 53 (4.8%) than other districts. Overall, clustered strains showed a varying distribution across districts and the districts such as Wondo Genet had all types of TB clusters. (Fig. 4).

Drug susceptibility profile

Drug susceptibility testing was carried out on 202 of 230 M. tuberculosis isolates for the first-line drugs: INH, RPM, EMB and for STM. A total of 29 (14.3%) isolates were found to be resistant to any of the drugs tested. Any resistance to one drug was most frequently observed for INH, 26 (12.8%) followed by ETB, 6 (2.9%) and STM, 4 (1.9%). The highest monoresistance was observed for INH, 22 (10.9%) followed by EMB, 6 (2.5%) and STM, 4 (0.5%). There was one case of monoresistance to RPM (0.3%). Combined drug resistance was observed for INH and EMB (1, 0.5%) and INH and STM (3, 1.4%). No MDR-TB was detected in the current study (Table 3).

Table 3
Resistance to first-line anti-TB drugs and to streptomycin

Total tested	Number	Percentage (%)	95% CI
	202		
Any resistance	29	14.3	9.83–19.96
Any Resistance to one drug			
Any INH	26	12.8	8.58–18.28
Any RPM	1	0.5	0.12–2.72
Any EMB	6	2.9	1.09–6.35
Any STM	4	1.9	0.45–4.99
Monoresistance			
INH only	22	10.9	6.92–16.02
RPM only	1	0.5	0.01–2.72
STM only	1	0.5	0.01–2.72
EMB only	5	2.5	0.80–5.68
Resistance to only drugs			
INH + RPM only	0	0	
INH + EMB only	1	0.5	0.01–2.72
INH + STM only	3	1.4	0.30–4.27
Resistance to four drugs			
INH + RPM + STM + EMB	0	0	

INH = Isoniazid, EMB = Ethambutol, RPM = Rifampicin, STM = Streptomycin

Genotyping of drug resistant strains

Genotyping of the drug-resistant strains showed that SIT 149 (T3-ETH) was the dominant strain (9/43) among the drug resistant isolates followed by SIT 53 (3/43) and SIT 390 (2/43).

Discussion

The study revealed a heterogeneous pool of *M. tuberculosis* strains with several clusters including lineage 7 strains circulating in southern Ethiopia. A high proportion of INH resistance was reported in the

study area and SIT 149 (T3-ETH) was the most dominant circulating strain in the study area including among drug-resistant cases. The high clustering of strains suggest the ongoing transmission of TB, including of drug-resistant TB in southern Ethiopia and calls for surveillance and wider monitoring of DST and improved control responses.

In the current study, the majority of the isolates (82%) belonged to the Euro-American lineage (L4) followed by East-Africa-Indian (L3), 14% and the Ethiopian lineage (L7), 4%. A recent study in southern Ethiopia (which was geographically close to our study) reported that 84% of the isolates were L4 and 3% of them were L3 (21). Studies from other parts of the country reported variable proportion of lineage types in different geographic areas of Ethiopia (12, 22, 23). Overall, L4 is more widely distributed and more predominant than all other lineages combined (24, 25). On the other hand, a higher proportion of L3 (25–35%) was reported in northern Ethiopia than elsewhere (12, 22). In general, it was noted that the geographic distribution and proportion of lineages varied across the country. The wider implication of this on the dynamics of the transmission of TB and drug resistance in the respective geographic localities is an area that has yet to be investigated well.

Lineage 7 accounted for 4% in the current study. One case of Lineage 7 was recently reported from the southern part of Ethiopia (21) and 6 cases (2%) from the southwest (26). Lineage 7 was first reported from Woldia area of Amhara region, Ethiopia with 13% of prevalence rate (12). Other studies from Amhara region (27, 28) have reported prevalence rates of 10% and 16%. So far, lineage 7 has been prominently reported from the northern part of Ethiopia. The additional report of lineage 7 in the current study suggests its broader occurrence, including in the southern parts of the country. Considering the pre-modern split of this lineage in the phylogenetic tree of *M. tuberculosis* and its localization to Ethiopia only, further investigations into its epidemiology would be of much interest.

In the current study, SIT 149 was the most common spoligotype (21%) circulating in the study area. Previous studies in Ethiopia have indicated that SIT149, also known as T3-ETH (29), ETH-3 and more recently as L4.2.ETH1 (25), is the most common spoligotype widely distributed in the country (24). It is also known to be more frequently associated with drug resistance than other spoligotype clusters (29). It is important that the distribution of this spoligotype is closely monitored and its drivers identified to better tailor control efforts.

Positioning of clustered strains using GIS mapping is helpful to describe the epidemiological links of *Mtb* strains in specific geographical localities (24). This information could be utilized to design targeted TB control measures. In the current study, geospatial analysis demonstrated variable distribution of strain clusters in the different districts of the study area suggesting areas affected with possible recent transmission. Spoligotyping may correctly identify *Mtb* complex in to various lineages and sub-lineages, however it is known to overestimate clustering of isolates due to its inferior discriminatory power compared to other genotyping techniques (10, 30, 31). In general, clustering of strains is a marker for recent transmission and can also serve to evaluate the performance of the TB control program (32) and help determine where to target interventions.

In the current study, 14% of the newly diagnosed TB patients were resistant to ≥ 1 first-line anti-TB drugs. In terms of the burden of the problem, this prevalence could be considered as significant. However, the result is lower than the prevalence rates reported in other parts of the country such as 23% (13) in Central Ethiopia and 23% in Eastern Ethiopia (6), but relatively higher than the prevalence rate of 11% reported in Northern Ethiopia (8) and 9% in Southern Ethiopia (33). Compared to a similar study conducted in this same study area in 2006 (34), the prevalence reported in this study (14%) is relatively lower than that of the previous one, 20%. The difference in the prevalence rates observed in different parts of the country could be due to differences in TB control program performance but also, more simply, due to study-related factors such as study design (population differences, methodology employed in the studies, sample size, study participant selection methods) or study periods. In addition to active case finding, it is essential for programs to build capacity for DST in order to implement recommended early detection and effective treatment of drug resistance to curb its spread (13).

INH monoresistance was 10.9% in the current study and it is comparable with reports from eastern Ethiopia, 9.5% (6), but lower than 13.2% from western Ethiopia (35) and higher than reports from central Ethiopia, 4.7% (13) and 2.3% in the previous report from the same study area (34). INH monoresistance is the first step towards anti-TB drug resistance and it is the common pathway for the development of MDR-TB (36). Therefore, the relatively high INH monoresistance that was observed in the current study should alert to the potential development of MDR TB in the study area and highlights the need for program based DST monitoring.

MDR-TB was not detected in the current study, even though two cases (0.9%) of MDR-TB were reported in a previous study in the same study area (34). Earlier studies have reported MDR-TB proportions of 1.1% (6), 1.2% (13) and 3.7% among newly diagnosed TB patients in eastern, central and northern parts of Ethiopia (8), respectively.

Linking strain typing data with data on drug resistance can be a useful way to monitor the spread of individual drug-resistant clones in communities (9). T3-ETH (SIT 149) was the most prevalent spoligotype (21%) among drug resistant strains in this series. Other studies have reported similar findings. Fifty percent (12/24) of the drug resistant *M. tuberculosis* isolates were SIT 149 in a collection dating from 2006–2010 (29). Similarly, in previous studies in the country (7, 14), T3-ETH (SIT 149) was associated with MDR-TB. In general, T3-ETH (SIT 149) is recognized as a predominant spoligotype cluster in Ethiopia most frequently associated with drug resistance, more so than other spoligotypes in the country (37). However, as it was indicated by Bekele and his colleagues (29), the observed association between T3-ETH (SIT 149) and development of drug resistance may not necessarily indicate that these strains are more prone to be drug-resistant but could rather be a consequence of their high prevalence in the population. This idea is also supported in a recent review (38) which showed that the correlation between genotypes and TB drug resistance was still uncertain. Further analysis on SIT149 identified genotype SIT149: A, a potential MDR-TB clone circulating in the Ethiopian highlands probably contributing to the spread of MDR-TB in the area that warrants further attention (29).

Limitations

The study had certain limitations. First, as our study participants were only newly diagnosed TB cases, it was not possible to assess the magnitude of drug resistant TB in the previously treated TB cases and the strain types among these groups. This has limited us from assessing the overall burden of drug resistant TB in the study area. Secondly, study participants were all patients seeking treatment at health facilities. Findings from such a selected population may not indicate the true burden of the problem at community level.

Conclusion

The study identified a heterogeneous pool of *M. tuberculosis* strains with several clusters including lineage 7 strains circulating in southern Ethiopia. No MDR-TB case was detected in the study area; however, the high proportion of INH monoresistance is a significant risk for the potential emergence of MDR-TB in the study area as INH monoresistance is the initial step towards anti-TB drug resistance and a common pathway to the development of MDR-TB. SIT 149 (T3-ETH) was the most dominant strain cluster circulating in the study area, including among drug resistant cases. The findings highlight the ongoing transmission of TB, including of drug resistant TB, in southern Ethiopia and call for surveillance and wider monitoring of DST, supported by geospatial analysis to monitor transmission trends and improve control responses.

Declarations

Acknowledgements

The authors thank Oromia Regional Health Bureau, Western Arisi zone Health Department, Southern Regional Health Bureau and Sidama Zone Health Department, Armauer Hansen Research Institute (AHRI) TB laboratory staff and Mr. Gebeyehu Assefa for their considerable assistance for the success of the work. We also sincerely thank Professor Henry M. Blumberg and Dr. Russell R. Kempker from Emory University School of Medicine in Atlanta, GA, USA for their invaluable assistance for the work.

Conflicts of interest: none declared.

Financial Disclosure Statement

The study was funded in part from the core AHRI budget (NORAD and SIDA grants) and the National Institutes of Health (NIH) Fogarty International Center Global Infectious Diseases grant entitled “Ethiopia-Emory TB Research Training Program” (D43TW009127) (AA).

Authors' contributions

YM: contributed to the conception and design of the study, acquisition of data and interpretation, and drafting and writing of the manuscript; YW, MA, DD: contributed to the design of the study and

supervision and revision of the manuscript; TH: contributed to data management and analysis; MH: contributed to geospatial analysis and revision of the manuscript; EH, MT and GH: contributed to laboratory work; AA: contributed to the design of the study and supervision, interpretation of data and reviewing the manuscript. All authors approved the final version of the manuscript.

References

1. WHO. Global Tuberculosis Report. Switzerland: World Health Organization, 2018.
2. Spence DP, Hotchkiss J, Williams CS, Davies PD. Tuberculosis and poverty. *Bmj*. 1993;307(6907):759-61.
3. Bartlett JG. Tuberculosis and HIV infection: partners in human tragedy. *The Journal of infectious diseases*. 2007;196 Suppl 1:S124-5.
4. Dye C, Williams BG. The population dynamics and control of tuberculosis. *Science*. 2010;328(5980):856-61.
5. EPHI. Second Round Anti-tuberculosis Drug Resistance Surveillance in Ethiopia. Addis Ababa, Ethiopia: Ethiopian Public Health Institute, 2014.
6. Seyoum B, Demissie M, Worku A, Bekele S, Aseffa A. Prevalence and Drug Resistance Patterns of *Mycobacterium tuberculosis* among New Smear Positive Pulmonary Tuberculosis Patients in Eastern Ethiopia. *Tuberculosis research and treatment*. 2014;2014:753492.
7. Agonafir M, Lemma E, Wolde-Meskel D, Goshu S, Santhanam A, Girmachew F, et al. Phenotypic and genotypic analysis of multidrug-resistant tuberculosis in Ethiopia. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease*. 2010;14(10):1259-65.
8. Tessema B, Beer J, Emmrich F, Sack U, Rodloff AC. First- and second-line anti-tuberculosis drug resistance in Northwest Ethiopia. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease*. 2012;16(6):805-11.
9. Jagielski T, Minias A, van Ingen J, Rastogi N, Brzostek A, Zaczek A, et al. Methodological and Clinical Aspects of the Molecular Epidemiology of *Mycobacterium tuberculosis* and Other *Mycobacteria*. *Clinical microbiology reviews*. 2016;29(2):239-90.
10. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *Journal of clinical microbiology*. 1997;35(4):907-14.
11. Zhang J, Abadia E, Refregier G, Tafaj S, Boschirola ML, Guillard B, et al. *Mycobacterium tuberculosis* complex CRISPR genotyping: improving efficiency, throughput and discriminative power of 'spoligotyping' with new spacers and a microbead-based hybridization assay. *Journal of medical microbiology*. 2010;59(Pt 3):285-94.
12. Firdessa R, Berg S, Hailu E, Schelling E, Gumi B, Erenso G, et al. *Mycobacterial* lineages causing pulmonary and extrapulmonary tuberculosis, Ethiopia. *Emerg Infect Dis*. 2013;19(3):460-3.

13. Tilahun M, Ameni G, Desta K, Zewude A, Yamuah L, Abebe M, et al. Molecular epidemiology and drug sensitivity pattern of *Mycobacterium tuberculosis* strains isolated from pulmonary tuberculosis patients in and around Ambo Town, Central Ethiopia. *PloS one*. 2018;13(2):e0193083.
14. Diriba B, Berkessa T, Mamo G, Tedla Y, Ameni G. Spoligotyping of multidrug-resistant *Mycobacterium tuberculosis* isolates in Ethiopia. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease*. 2013;17(2):246-50.
15. WHO. Laboratory services in tuberculosis control. Part III: Culture. Geneva, Switzerland: World Health Organization, 1998.
16. WHO. Guideline for surveillance of drug resistance in tuberculosis. Geneva: World Health Organization, 2003.
17. Wedajo W, Schon T, Bedru A, Kiros T, Hailu E, Mebrahtu T, et al. A 24-well plate assay for simultaneous testing of first and second line drugs against *Mycobacterium tuberculosis* in a high endemic setting. *BMC research notes*. 2014;7:512.
18. Parsons LM, Brosch R, Cole ST, Somoskovi A, Loder A, Bretzel G, et al. Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis. *Journal of clinical microbiology*. 2002;40(7):2339-45.
19. Shabbeer A, Cowan LS, Ozcaglar C, Rastogi N, Vandenberg SL, Yener B, et al. TB-Lineage: an online tool for classification and analysis of strains of *Mycobacterium tuberculosis* complex. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases*. 2012;12(4):789-97.
20. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of biomedical informatics*. 2009;42(2):377-81.
21. Molina-Moya B, Agonafir M, Blanco S, Dacombe R, Gomgnimbou MK, Spinasse L, et al. Microbead-based spoligotyping of *Mycobacterium tuberculosis* from Ziehl-Neelsen-stained microscopy preparations in Ethiopia. 2018;8(1):3987.
22. Nuru A, Mamo G, Worku A, Admasu A, Medhin G, Pieper R, et al. Genetic Diversity of *Mycobacterium tuberculosis* Complex Isolated from Tuberculosis Patients in Bahir Dar City and Its Surroundings, Northwest Ethiopia. *BioMed research international*. 2015;2015:174732.
23. Bedewi Z, Mekonnen Y, Worku A, Medhin G, Zewde A, Yimer G, et al. *Mycobacterium tuberculosis* in central Ethiopia: drug sensitivity patterns and association with genotype. *New microbes and new infections*. 2017;17:69-74.
24. Tulu B, Ameni G. Spoligotyping based genetic diversity of *Mycobacterium tuberculosis* in Ethiopia: a systematic review. *BMC infectious diseases*. 2018;18(1):140.
25. Comas I, Hailu E, Kiros T, Bekele S, Mekonnen W, Gumi B, et al. Population Genomics of *Mycobacterium tuberculosis* in Ethiopia Contradicts the Virgin Soil Hypothesis for Human Tuberculosis in Sub-Saharan Africa. *Current biology : CB*. 2015;25(24):3260-6.

26. Tadesse M, Abebe G, Bekele A, Bezabih M, de Rijk P, Meehan CJ, et al. The predominance of Ethiopian specific *Mycobacterium tuberculosis* families and minimal contribution of *Mycobacterium bovis* in tuberculous lymphadenitis patients in Southwest Ethiopia. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases*. 2017;55:251-9.
27. Biadlegne F, Merker M, Sack U, Rodloff AC, Niemann S. Tuberculous Lymphadenitis in Ethiopia Predominantly Caused by Strains Belonging to the Delhi/CAS Lineage and Newly Identified Ethiopian Clades of the *Mycobacterium tuberculosis* Complex. *PloS one*. 2015;10(9):e0137865.
28. Yimer SA, Norheim G, Namouchi A, Zegeye ED, Kinander W, Tonjum T, et al. *Mycobacterium tuberculosis* lineage 7 strains are associated with prolonged patient delay in seeking treatment for pulmonary tuberculosis in Amhara Region, Ethiopia. *Journal of clinical microbiology*. 2015;53(4):1301-9.
29. Bekele S, Derese Y, Hailu E, Mihret A, Dagne K, Yamuah L, et al. Line-probe assay and molecular typing reveal a potential drug resistant clone of *Mycobacterium tuberculosis* in Ethiopia. *Tropical diseases, travel medicine and vaccines*. 2018;4:15.
30. Garcia de Viedma D, Mokrousov I, Rastogi N. Innovations in the molecular epidemiology of tuberculosis. *Enfermedades infecciosas y microbiologia clinica*. 2011;29 Suppl 1:8-13.
31. Singh J, Sankar MM, Kumar P, Couvin D, Rastogi N, Singh S. Genetic diversity and drug susceptibility profile of *Mycobacterium tuberculosis* isolated from different regions of India. *The Journal of infection*. 2015;71(2):207-19.
32. Small PM, Hopewell PC, Singh SP, Paz A, Parsonnet J, Ruston DC, et al. The epidemiology of tuberculosis in San Francisco. A population-based study using conventional and molecular methods. *The New England journal of medicine*. 1994;330(24):1703-9.
33. Wondale B, Medhin G, Abebe G, Tolosa S, Mohammed T, Teklu T, et al. Phenotypic and genotypic drug sensitivity of *Mycobacterium tuberculosis* complex isolated from South Omo Zone, Southern Ethiopia. *Infection and drug resistance*. 2018;11:1581-9.
34. Arega D. Epidemiology and Drug Resistance Pattern of Mycobacterial Isolates among HIV positive and HIV negative TB patients using Conventional and Molecular Methods in South east Ethiopia: Addis Ababa University; 2007 (un-published).
35. Abebe G, Abdissa K, Abdissa A, Apers L, Agonafir M, de-Jong BC, et al. Relatively low primary drug resistant tuberculosis in southwestern Ethiopia. *BMC research notes*. 2012;5:225.
36. Cohen KA, Abeel T, Manson McGuire A, Desjardins CA, Munsamy V, Shea TP, et al. Evolution of Extensively Drug-Resistant Tuberculosis over Four Decades: Whole Genome Sequencing and Dating Analysis of *Mycobacterium tuberculosis* Isolates from KwaZulu-Natal. *PLoS medicine*. 2015;12(9):e1001880.
37. Yimer SA, Norheim G, Namouchi A, Zegeye E, Kinander W, Tønjum T, et al. *Mycobacterium tuberculosis* Lineage 7 Strains Are Associated with Prolonged Patient Delay in Seeking Treatment for Pulmonary Tuberculosis in Amhara Region, Ethiopia 2015.

38. Panwalkar N, Chauhan DS, Desikan P. Spoligotype defined lineages of Mycobacterium tuberculosis and drug resistance: Merely a casual correlation? Indian journal of medical microbiology. 2017;35(1):27-32.

Figures

Figure 1
Map of the study area (East Arisi Zone districts, Oromia and Wondo Genet of Sidama Zone)

Figure 2

Study Diagram. DST-drug susceptibility testing, RD9-region of difference-9

Figure 3

Spatial distribution of TB lineages, West Arisi zone and Wondo Genet districts

Figure 4

Spatial distribution of clustered strains by district, West Arisi zone and Wondo Genet districts

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Assentformforchildren.pdf](#)
- [Parentsguardiansconsent.pdf](#)